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Redescription of *Ophiotaenia hylae* Johnston, 1912 (Eucestoda: Proteocephalidea), parasite of *Litoria aurea* (Amphibia: Hylidae) from Australia

Alain DE CHAMBRIER
Muséum d'histoire naturelle, Département des Invertébrés, PO Box 6434,
CH-1211 Geneva 6, Switzerland. E-mail: alain.dechambrier@mhn.ville-ge.ch

Redescription of *Ophiotaenia hylae* Johnston, 1912 (Eucestoda: Proteocephalidea), parasite of *Litoria aurea* (Amphibia: Hylidae) from Australia. - Type material of the proteocephalidean cestode *Ophiotaenia hylae* Johnston, 1912 is redescribed. It is characterised by a globular scolex with uniloculate suckers, a prominent apical organ covered by spiniform microthriches and containing round to oblong cells of finely granular cytoplasm, and by the internal longitudinal musculature composed by 4-5 dorsal and 4-5 ventral bundles of fibres. A similar taxon, *Ophiotaenia* sp. from a closely related host species, *Litoria moorei*, is also studied and compared.

Key-words: Eucestoda - Proteocephalidea - *Ophiotaenia hylae* - *Litoria aurea* - *Litoria moorei* - Hylidae - Australia.

INTRODUCTION

Since its original description in 1912, nobody made a redescription of *O. hylae*. Prudhoe & Bray (1982, p. 33, figs 8a, b, c) only published drawings of *Ophiotaenia hylae* (BMNH 1968.4.19.1-15) from *Litoria (Hyla) moorei*, Cannington, Australia. This material has been examined and is here considered as belonging to *Ophiotaenia* sp. (see below) and not to *O. hylae* Johnston. New material of the true *O. hylae* was unavailable due to the extreme scarcity of its host, *Litoria (Hyla) aurea*. This species is actually considered threatened with extinction in Australia (Pyke *et al.*, 2002; Pyke, 2002). I had the opportunity to study Johnston's type material deposited in different Australian museums (Queensland Museum, Brisbane; South Australian Museum, Adelaide). This allowed me to redescribe this material, to add new information to the original description and clarify its taxonomic status.

MATERIAL AND METHODS

The worms, conserved in museum collection in alcohol, were stained with Weigert's haematoxylin solution, dehydrated in an ethanol series, cleared with Eugenol (clove oil), and mounted in Canada balsam. Pieces of strobila were embedded in paraffin wax, cross sectionned (thickness 12-15 μm), stained with Weigert's haema-

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toxylin and counterstained with 1% eosin B according to the method recently published by de Chambrier (2001). All measurements are given in micrometres unless otherwise stated.

Abbreviations used in descriptions: x = mean, n = number of measurements, CV = coefficient of variability (%), OV = ovary width to proglottis width ratio, PG = position of the genital pore in relation to proglottis length (%), PC = cirrus pouch length to proglottis width ratio (%), JNT = Johnston original description; BMNH = Natural History Museum, London; MHNG = Natural History Museum, Geneva; INVE = Geneva Museum, Invertebrates Collection; SAM = South Australian Museum, Adelaide; QM = Queensland Museum, Brisbane.

RESULTS

Ophiotaenia hylae Johnston Figs 1-6

Ophiotaenia hylae Johnston, 1912: 63.
Batrachotaenia hylae; Rudin, 1917: 366.
Batrachotaenia hylae; Freze, 1965: 385.

Type host: *Litoria aurea* (Lesson, 1829) (Amphibia: Hylidae).

Material studied: Syntype material of *Ophiotaenia hylae*: 1 slide V 4141 (SAM 44141); S 689 (SAM 20689), four slides: a) 2 immature pieces, one with scolex; b) 1 immature piece, 9 mm; c) 1 gravid proglottis, 1 mm, bad conservation state; d) 11 gravid proglottides, 15 mm. One immature specimen with a scolex, G 16/423, QM, from *Hyla aurea*, Sydney, NSW, 37°41'S, 144°40'E. Other material: In one separate box containing a lot of Johnston's original material, 1 slide with 12 immature pieces, with one scolex, SAM 28407.

Site of infestation: Intestine.

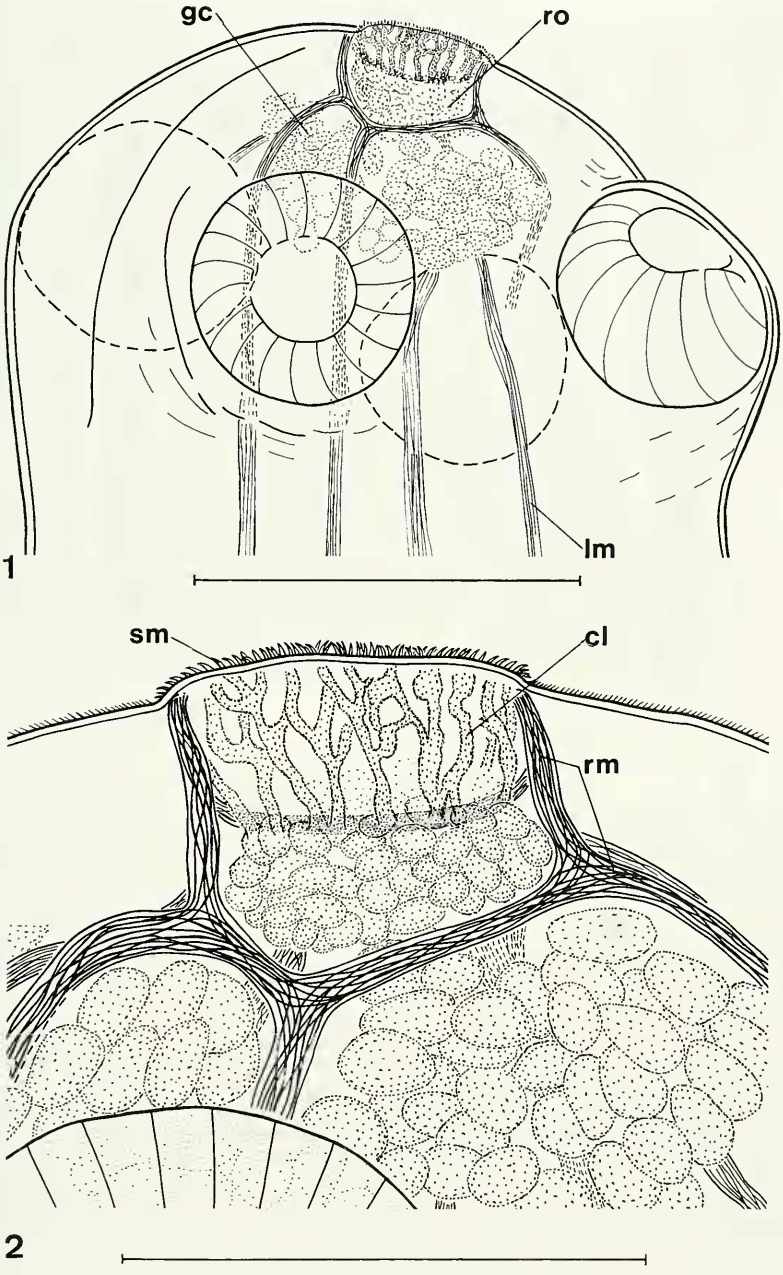
Type-locality: Neighbourhood of Sydney, NSW, Australia.

Redescription (based on syntypes and Johnston's original material)

Proteocephalidea, Proteocephalidae. Testes, ovary, uterus diverticles in medulla, uterine stem cortical. In the whole mounted syntype material, one fragment 18 mm long. Strobila acraspedote, anapolytic, consisting of 33 immature and mature proglottides (JNT = 60 mm long). Immature proglottides 540-635 long and 405-500 wide, mature proglottides 695-750 long and 710-865 wide, gravid proglottides 1250-1540 long and 865-920 wide (Figs 3, 4). Tegument thick and wrinkled in mature proglottides. Presence of numerous small dorso-ventral muscles.

Scolex 340-390 (JNT = 320) in diameter (Figs 1-2), covered by small dense microtriches about 1 long, suckers 130-135 (JNT =110) in diameter. Apical organ, 65-80 in diameter, covered by small dense spiniform microtriches 2-3 long (Fig. 2) above a network of small and poorly defined canals filled with a granular content, and ending beneath tegument surface. Presence of small retractor musculature at the margin of the apical organ (Fig. 2). Beneath the apical organ, a concentration of cells with a finely granular cytoplasm is present in two zones, one just beyond the apical organ and another made of twice bigger cells situated at the level of the suckers (Fig. 2). Longitudinal internal musculature dense, formed by 4-5 thick bundles of fibres on both dorsal and ventral sides (Fig. 5).

Ventral and dorsal osmoregulatory canals between vitelline follicles and testes, crossing cirrus pouch at level of its two/third part (Fig. 3). Ventral canal, twice the



FIGS 1-2

Ophiotaenia hylae Johnston, 1912. Syntype, 20689 SAM. 1. Scolex, general view. 2. Detailed view of the apical organ region. *Abbreviations*: cl, small canals- filled with granular content; gc, gland cells; lm, internal longitudinal musculature; rm, retractor muscles; ro, rostellum-like apical organ; sm, spiniform microtriches (hooklets). Scale-bars: 1 = 200 μm; 2 =100 μm.

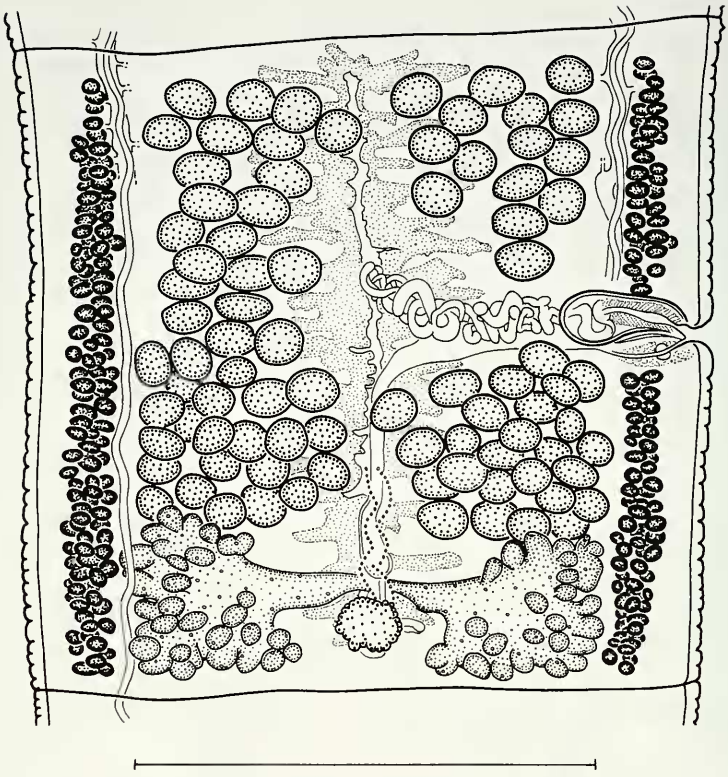
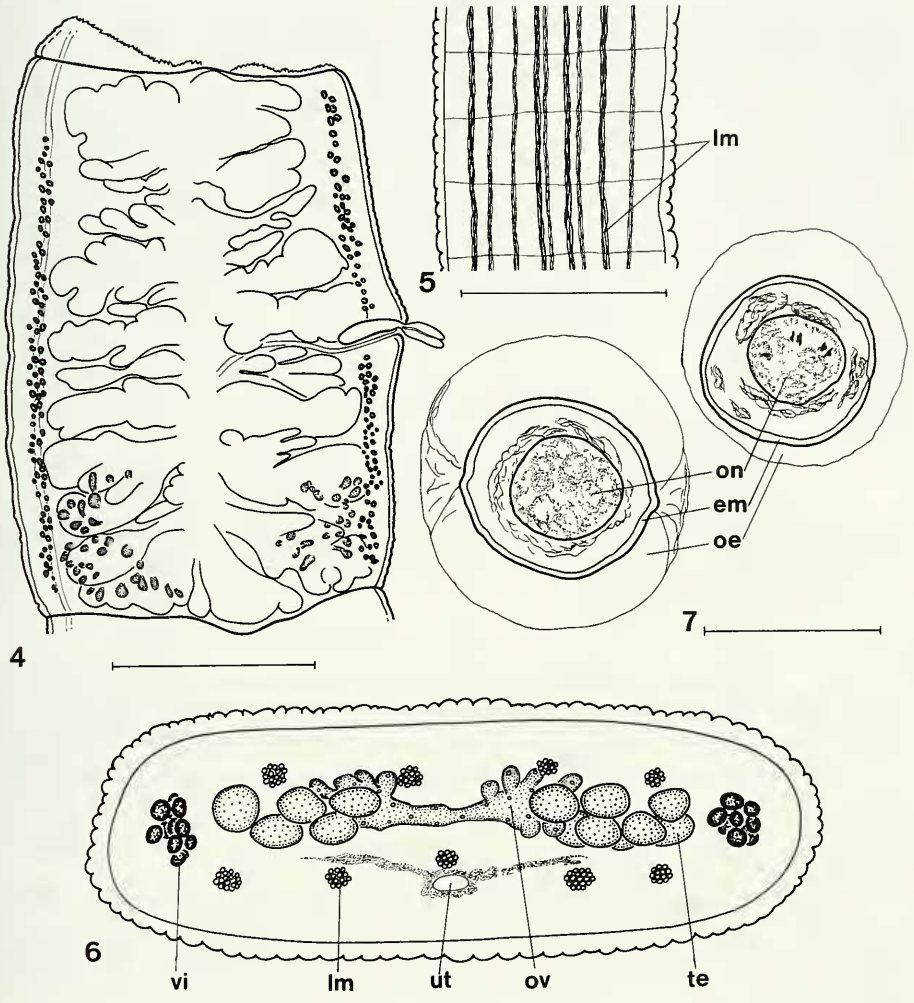


FIG. 3

Ophiotaenia hylae Johnston, 1912. Syntype, 44141 SAM, dorsal view of a mature proglottis. Scale-bar: 500 μm.

diameter of the dorsal canal, with narrow secondary canals directed externally ventrally. Testes 74-106 in number (x = 86, n = 12, CV = 13%, JNT = numerous) in two dorsal field, with tendency to converge anteriorly and posteriorly, in one or two layers dorsally, not reaching laterally to vitelline follicles (Fig. 3), 35-60 in diameter. Testes 15-22 preporal, 16-25 postporal and 38-53 aporal in number. Testes degenerated in gravid proglottides (Fig. 4).

Genital pores irregularly alternating, opening between 44 and 55 % (n = 12, CV = 7%) of proglottis length. Small genital atrium present. Cirrus pouch pyriform, 115-145 long (JNT 140). PC = 17-19% (x = 18%, n = 11, CV = 5%). Cirrus occupying up to 70% of cirrus pouch length. Evaginated cirrus covered by numerous minute spiniform microtriches, 2-3 long. Vagina anterior (38%) or posterior (62%) to cirrus pouch (JNT = antero-ventrally), with a small subterminal vaginal sphincter (Fig. 3). When anterior, passing ventrally to the cirrus pouch. Mehlis glands 60-85 in diameter. Vas deferens coiled, between base of cirrus pouch and median part of proglottis, rarely extending beyond body midline in mature and premature proglottides, extending anteriorly.



FIGS 4-7

4-6. *Ophiotaenia hylae* Johnston, 1912. Syntype material, 20689 SAM. 4. Scheme of dorsal view of a gravid proglottis showing the uterine diverticle development. 5. Scheme of the internal longitudinal musculature at level of immature proglottides. 6. Scheme of a cross section showing the disposition of genital organs related to the internal longitudinal musculature. 7. *Ophiotaenia* sp. from *Litoria moorei*. BMNH 1968.4.19.1-15, eggs drawn in distilled water. *Abbreviations*: em, embryophore; lm, internal longitudinal musculature; oe, outer envelope; on, oncosphere; ov, ovary; te, testes; ut, uterus; vi, vitellaria; Scale-bars: 4 = 500 μm; 5 = 250 μm; 6 = no scale; 7 = 20 μm.

Ovary bilobate, medullary, folliculate, with numerous dorsal outgrowths (Figs 3, 7). OV = 68-71% (x = 70%, n = 11, CV = 2%). Vitelline follicles, in two lateral bands, occupying porally 91-97% of proglottis length, and aporally 94-97% of proglottis length (Fig. 3).

Primordium of uterine stem cortical, already present in immature proglottides, with diverticles in medulla. Formation of uterus of type 2 (see de Chambrier *et al.*, 2004): in immature proglottides, chromophil cells concentrated laterally on both sides of uterine stem; in the first mature proglottides, lateral ramified digitations without a lumen, occupying at this stage already about 35% of proglottis width; in gravid proglottides, lateral diverticles occupying up to 91% of gravid proglottis width. Uterus with 10-17 (JNT = numerous) lateral medullar ramified diverticles on each side (Fig. 4) and one or sometimes several ventral apertures as described for *Crepidobothrium* spp (de Chambrier, 1989a, b). Eggs, measured in whole preparations, with oncosphere 11-12 in diameter (JNT = 7.5-11), hooklets 5-6 long; embryophore 13-14 in diameter (JNT = 15-19); outer envelope 60-75 in diameter.

Ophiotaenia sp. Figs 7-10

Proteocephalus hylae; Prudhoe & Bray, 1982: 33, Figs 8a, b, c.
[Not *Ophiotaenia hylae* Johnston].

Host: *Litoria moorei* (Copland, 1957) (Amphibia : Hylidae).

Locality: Neighbourhood of Perth (Cannington and Darlington), W.A., Australia.

Material studied: 9 whole mount preparations and material in alcohol (from where SEM microphotographs come from) ex *Litoria moorei*, Cannington, Western Australia, 17.04.1966: BMNH 1968.4.19.1-15; 1 whole mount preparation SAM 21402, Darlington, W.A., 12.11.1980.

Site of infestation: Intestine.

DESCRIPTION

Proteocephalidea, Proteocephalidae. Testes, ovary, uterus diverticles in medulla, uterine stem cortical. Strobila acraspedote, anapolytic. Tegument thick and wrinkled in mature proglottides. Presence of numerous small dorso-ventral muscles.

Scolex 260-340 (n = 4, x = 290) in diameter, suckers 105-130 in diameter (Fig. 9). Apical organ, 50-80 in diameter, covered by small dense spiniform microtriches (Fig. 10). Longitudinal internal musculature dense, formed by 4-5 thick bundles of fibres on both dorsal and ventral sides (Fig. 8).

Ventral and dorsal osmoregulatory canals crossing cirrus pouch at its middle part, situated between vitelline follicles and testes (Fig. 8). Ventral canal, twice the diameter of the dorsal canal, with numerous narrow secondary canals directed externally.

Testes 46-76 in number (x = 59, n = 30, CV = 14%) in two dorsal field, with tendency to converge anteriorly and posteriorly, in one or two layers dorsally, not reaching laterally to vitelline follicles (Fig. 8), 50-80 in diameter. Testes 14-26 preporal, 6-18 postporal and 23-44 aporal in number. Testes degenerated in gravid proglottides.

Genital pores irregularly alternating, opening between 46 and 57 % (n = 17, CV = 6%) of proglottis length. Small genital atrium present. Cirrus pouch pyriform, 175-215 long, PC = 27-33% (x = 29%, n = 17, CV = 4%). Cirrus occupying up to 85% of cirrus pouch length. Vagina anterior (54%) or posterior (46%) to cirrus pouch, with a sub-terminal vaginal sphincter (Fig. 8). When anterior, passing ventrally to the cirrus pouch. Mehlis glands 45-65 in diameter. Vas deferens coiled, between base of cirrus pouch and median part of proglottis, often extending beyond body midline in mature and premature proglottides, extending anteriorly.

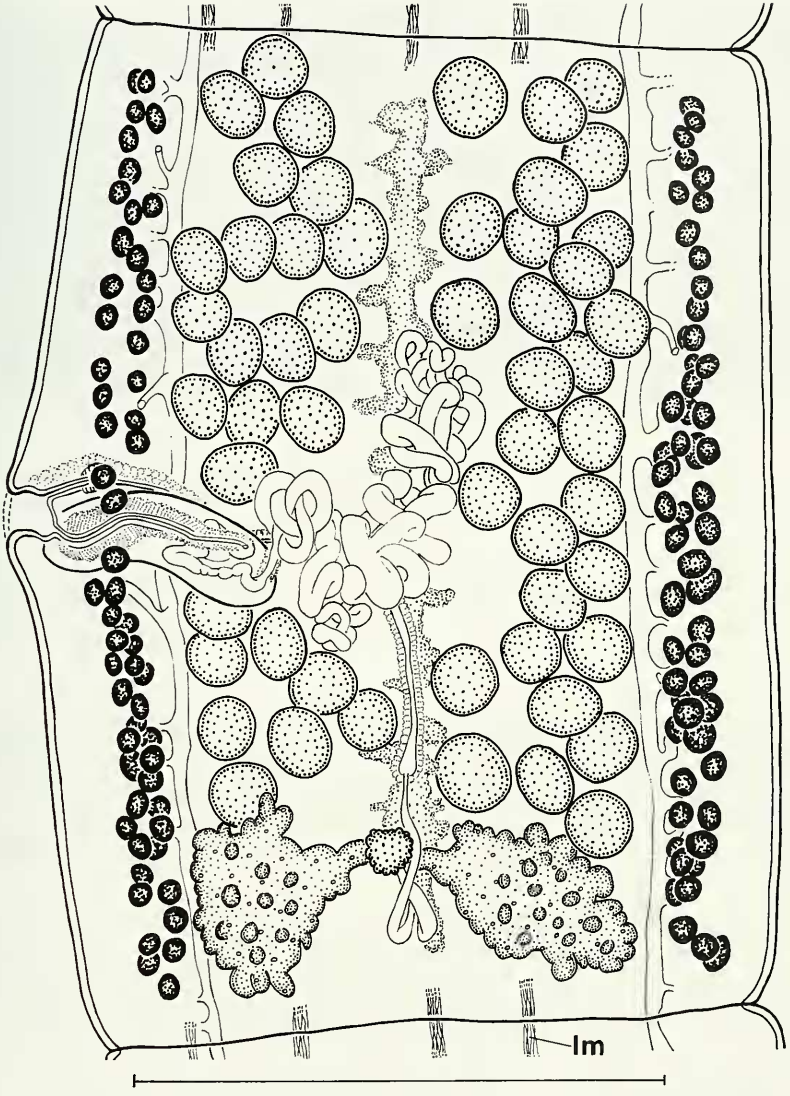
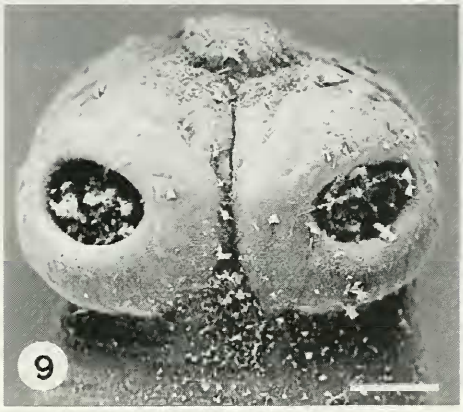


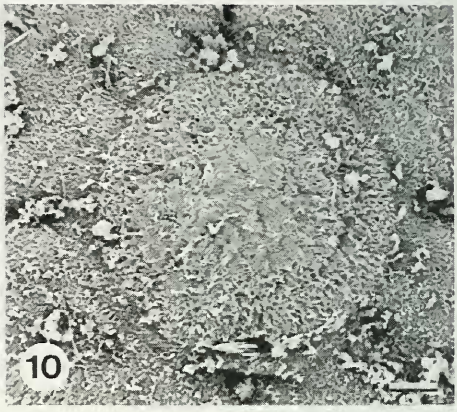
FIG. 8

Ophiotaenia sp. from *Litoria moorei*. 21402 SAM. Mature proglottis, dorsal view. Note the secondary canals ending beneath the tegument. Scale-bar: 500 μm.

Ovary bilobate, medullary, folliculate, with dorsal outgrowths. OV = 55-63% (x = 60%, n = 17, CV = 3%) (Fig. 8). Vitelline follicles, in two lateral bands, occupying porally 87-91% of proglottis length and aporally 86-88% of proglottis length.

Primordium of uterine stem cortical, already present in immature proglottides, with diverticles in medulla. Formation of uterus of type 2 (see de Chambrier *et al.*, 2004).





FIGS 9-10

Ophiotaenia sp., BMNH 1968.4.19.1-15, from *Litoria moorei*. Scanning electron micrographs of the scolex. 9. Dorsoventral view. 10. Apical view, detail of the apical organ. Scale-bars: 9 = 50 μm; 10 = 10 μm.

Eggs, measured in distilled water, with oncosphere 12-18 in diameter, hooklets 5-9 long; embryophore 18-23 in diameter; outer envelope up to 55 in diameter (Fig. 7).

REMARKS

This taxon is similar to *Ophiotaenia hylae* on the basis of the following characters: similar apical organ, position of the genital pore, presence of 4-5 dorsal and 4-5 ventral longitudinal internal bundles of musculature. It differs from it by the number of testes (46-76 versus 74-106), by the cirrus pouch length/width of proglottis ratio (27-33% versus 17-19%), and by the ovary width / proglottis width ratio (55-63% versus 68-71%).

Although these observations suggest that it could belong to a new distinct species, the material studied is fragmented and not in suitable conditions for an accurate description. The scolex particularly is badly fixed. In order to confirm that it represents a new species, it would be necessary to collect new material from *Litoria moorei*.

DISCUSSION

Johnston (1912) situated the ovary and the vitelline follicles of *Ophiotaenia hylae* in the cortex. My observations show the ovary to be clearly medullary (see scheme, Fig. 6). As for the vitelline follicles, their position is difficult to assess as there are no clear lateral muscle bundles (Fig. 6). The uterus stem is cortical with further development of diverticles into the medulla (Fig. 6). Contrary to the opinion of Johnston (1912, p. 64), the uterus does not arise as "a thin duct..." but is clearly of the type 2 of uterine development as described by de Chambrier *et al.* (2004). According to Johnston (1912), the vagina is situated anterior and ventral to the cirrus pouch. I observed a position mainly posterior (62%) of the vagina. I also observed a small sub-

terminal vaginal sphincter, secondary canals emerging from the ventral osmoregulatory canal ending under the tegument and the position of vas deferens which extends anteriorly (see Fig. 3).

The structure of the internal longitudinal musculature is also uncommon within the Proteocephalidea. It is composed by 4-5 dorsal and 4-5 ventral powerful isolate bundles of musculature easy to observe in immature and mature proglottides but less so in gravid proglottides. Given the stability of this character, the number of bundle in mature proglottides could be discriminant at the specific level as I already proposed for *Crepidobothrium* species (de Chambrier, 1989b, p. 369).

The apical organ is peculiar because of the presence of small spiniform hooklets covering its surface, retractor-like muscles and network of small canals surrounding it. This morphology shows some similarities with that of the Gangesiinae and looks intermediate between the apical organs found in the *Nomimoscolex piraeeba* aggregate (Zehnder *et al.*, 2000) and those in the Gangesiinae (de Chambrier *et al.*, 2003). To my knowledge, no other Proteocephalidea have this kind of apical organ. It would be interesting to analyse the two Australian *Ophiotaenia* species described in the present paper using DNA sequences, and compare them with the taxa cited above in order to see if their respective apical organs could represent a possible evolutionary trend or if this structure is homoplastic.

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